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Evaluation of Alternate Pretreatment and Biomass Fractionation -Ammonia Recycled Percolation Precess

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Abstract

An ammonia based biomass pretreatment method termed as Ammonia Recycled Percolation process was investigated. This process is characterized by: 1) aqueous ammonia is used as a pretreatment reagent, 2) a packed-bed flow through type reactor (percolation reactor) is used in recirculation mode, 3) ammonia is continuously recycled. The experimental data on hybrid poplar indicate that the ARP is a highly effective pretreatment method. The digestibility of the best-case ARP sample approached that of filter paper. The extent of delignification in the ARP process was in the range of 23-63%. The ARP process solubilized significant amount of the xylan fraction into the pretreatment effluent yet leaving the glucan fraction intact. It has also been demonstrated that pretreatment with pure water at elevated temperatures (autohydrolysis) prior to the ARP can selectively remove most of the hemicellulose content in the biomass. Drying of the pretreated biomass before enzymatic hydrolysis brought about a detrimental effect on the digestibility due to recrystallization of cellulose. The SEM examination of the solid samples revealed that the ARP induces considerable morphological changes reflecting the increase in pore size and porosity. The overall material balance of ammonia has shown that the recovery factor of ammonia in the ARP process is in excess of 99%.

Introduction

As an alternative pretreatment method environmentally compatible and capable of removing the lignin and other nonfermentable fraction from the fermentable carbohydrates, an ammonia based pretreatment method is proposed and being investigated for its key technical elements. The proposed method is termed as **Ammonia Recycled Percolation** Process, or **ARP** Process. Its main technical features are: 1) aqueous ammonia (and/or ammonium hydroxide) is used as the pretreatment reagent, 2) a packed-bed flow through type (percolation) reactor is employed and operated under a recirculation mode, 3) ammonia is continuously regenerated and recycled during the process.

Ammonia is a proven delignification reagent. It carries reactive characteristics strong enough to cause significant changes in the biomass other than delignification. Use of ammonia can bring about not only the pretreatment effects but also the fractionation of biomass, especially separation of lignin from biomass. This is an important attribute in ammonia pretreatment for several important reasons. First, the lignin content of the pretreated biomass can be lowered to any desired level, thus increase the efficiency in enzyme use during hydrolysis. Second, as is the case with most alkaline treatment, ammonia treatment does not cause significant loss of carbohydrates. Third, the lignin generated in this process is sulfur-free and sodium-free unlike the ones generated from pulping processes. It is generally of high quality and commands high byproduct credit. It is quite conceivable that the uncontaminated lignin can be marketed for applications in various areas including fuel additives, polymer applications, adhesives, and asphalt extender. High volatility of ammonia in comparison to water makes it easy to separate from aqueous mixture. A straight batch evaporation is sufficient to remove all of ammonia content in the mixture. Therefore the unbound ammonia is easily recovered and recycled, a key feature in the ARP process scheme. Whether the hemicellulose is retained after pretreatment or not may play on a significent role in the peocess design. It is well known that "cellulase" enzyme exhibits considerable xylanase activity as well as cellulase activity. Therefore hydrolysis of cellulose and hemicellulose occur simultaneously by the action of "cellulase". For certain microbial processes are designed to utilize both glucose and pentoses, including SSF for butanol production, simultaneous fermentation and isomerization of xylose (SFIX). If xylose processing is a separate one, then additional acid hydrolysis of hemicellulose would be required.

Ammonia, although generally considered toxic in the form of vapor, is not an acute health hazard. It is one of the components that exists in human body. It is also one of the most heavily used industrial commodity chemicals. To our knowledge, there is no evidence of harmful byproduct formation from ammonia-lignin-carbohydrate interaction at elevated temperatures. Ammonia is inexpensive to the extent that it is used for synthesis of N-fertilizer (urea). The current price of ammonia is $108/Ton whereas sulfuric acid is $75/Ton . <u>On molar basis</u> ammonia costs only <u>one-fourth</u> the price of sulfuric acid.

We must also address the potential problems associated with the ARP process. The most important one has to do with consumption of ammonia due to interaction with lignin and neutralization by acetates and other buffering components known to exist in biomass. The nature of reactions involving ammonia and lignocellulosics is quite complex. We know that most of the ammonia input to the process is recovered and reused under the concept of this process design. It is difficult to predict how much ammonia is irreversibly consumed during the pretreatment process. It is certainly a major item to be investigated in this study.

From a process viewpoint, there is a concern as to the high pressure condition that may develop due to the highly volatile nature of ammonia. However, within the range of expected reaction conditions of 140°C-180°C, and 5 - 10% NH_3, the upper limit of the pressure is about 20 Kg/cm^2 or 290 psi. It is somewhat higher than a normal pulp mill digester pressure, but certainly within a manageable range. Ammonia is far less corrosive than sulfuric acid at high temperature. Overall, it does not present a major technical problem to design an operate a process of this nature.

The concept of percolation process applies well to this pretreatment design. The distinctive feature of the percolation process in comparison to a straight batch process is that the process stream is continuously fed and withdrawn from the reactor. In connection with the biomass pretreatment, it offers unique advantage that the lignin and other extraneous components are separated from the biomass structure. This prevents recondensation of lignin within the biomass. It also eliminates the need for washing of pretreated biomass which will have a significant bearing in the operation cost (especially in the ammonia regeneration step). Percolation reactor system has a wide application in pretreatment process. It can be easily adapted to other pretreatment methods including dilute acid pretreatment (the base case design in NREL). It is most likely that the percolation reactor system (or a variation of it) would be installed at NREL for pilot scale study on pretreatment in any event. If the proposed system is to be tested in NREL, it would require only a minor modification of the existing hardware.

The technical objective of this subcontract (as specified in the Statement of Work issued by NREL) is to identify and develop pretreatment approaches that could improve on the performance and cost of ethanol production over that now observed for dilute sulfuric acid technology. The emphasis is on those aspects that will improve the economics and environmental compatibility. Technologies are sought that can remove the lignin and other nonfermentable fraction from the fermentable carbohydrates.

With this understanding, the first year program of this subcontract was put in force with emphasis on the study on the technical feasibility in the ARP process. This report summarizes the first year research progress of this subcontract.

Materials/Methods

Materials

Hybrid poplar milled from whole tree (including bark) to the nominal size of 1/16 inch -60 mesh was supplied from NREL and used throughout this study. The cellulase enzyme, Cytolase CL, Lot No. 17-92262-09, was supplied from Environmental Biotechnology, Inc. The specific activity of the enzyme as determined by the supplier is as follows: Filter paper activity = 103 FPU/mL, β-G activity = 88.9 pNPGU/mL, Endo-G activity = 269 CMCU/mL. The microorganism, Saccharomyces cerevisiae, strain No. NREL-YST-02B, was also provided by NREL and used in the simultaneous saccharification and fermentation studies.

Experimental Set-Up and Operation

The ARP pretreatment involve treating biomass with ammonium hydroxide solution at temperatures above 150°C. The batch experimental setup for the ARP process is shown in Figure 1. The system is comprised of aqueous ammonia reservoir, pump, sand bath, packed bed reactor, and liquid holding tank/back pressure vessel. The reactor was constructed out of SS-316 tubing, 5/8 inch OD x 6 inch L (33 cm^3 of internal reactor volume). It is flanged and screen sealed at both ends. An autoclave (600 ml, Parr Instrument) was used as a liquid holding tank. It was connected to a nitrogen cylinder to apply back pressure to the system. A positive displacement pump (Metering Minipump, LDC) was used to deliver aqueous ammonia against reactor pressure. All the connecting lines and fittings were of SS-316 grade. Aqueous ammonia was pumped through a preheating coil to the bottom section of the reactor prepacked with biomass substrates. The preheating coil and the reactor were submerged and heated in the sand bath. The aqueous ammonia was passed through the reactor and collected at the liquid holding tank. The flow rate of aqueous ammonia was controlled by pump speed and monitored by flow gauge and direct reading of burette. Temperature of the reactor was controlled by adjusting the sand bath electrical heater/controller. The pressure of the system was controlled by the regulator of the nitrogen cylinder. The second reactor constructed out of 316 tubing, 3/4 inch OD x 10 inch L (73 cm^3 empty reactor volume) was also used to produce enough sample for SSF experiment. Temperature programmable oven of GC was also used in place of the sand bath.

Biomass feed was mixed with water and placed in a ultrasound bath (Mettler Electronics Co.)

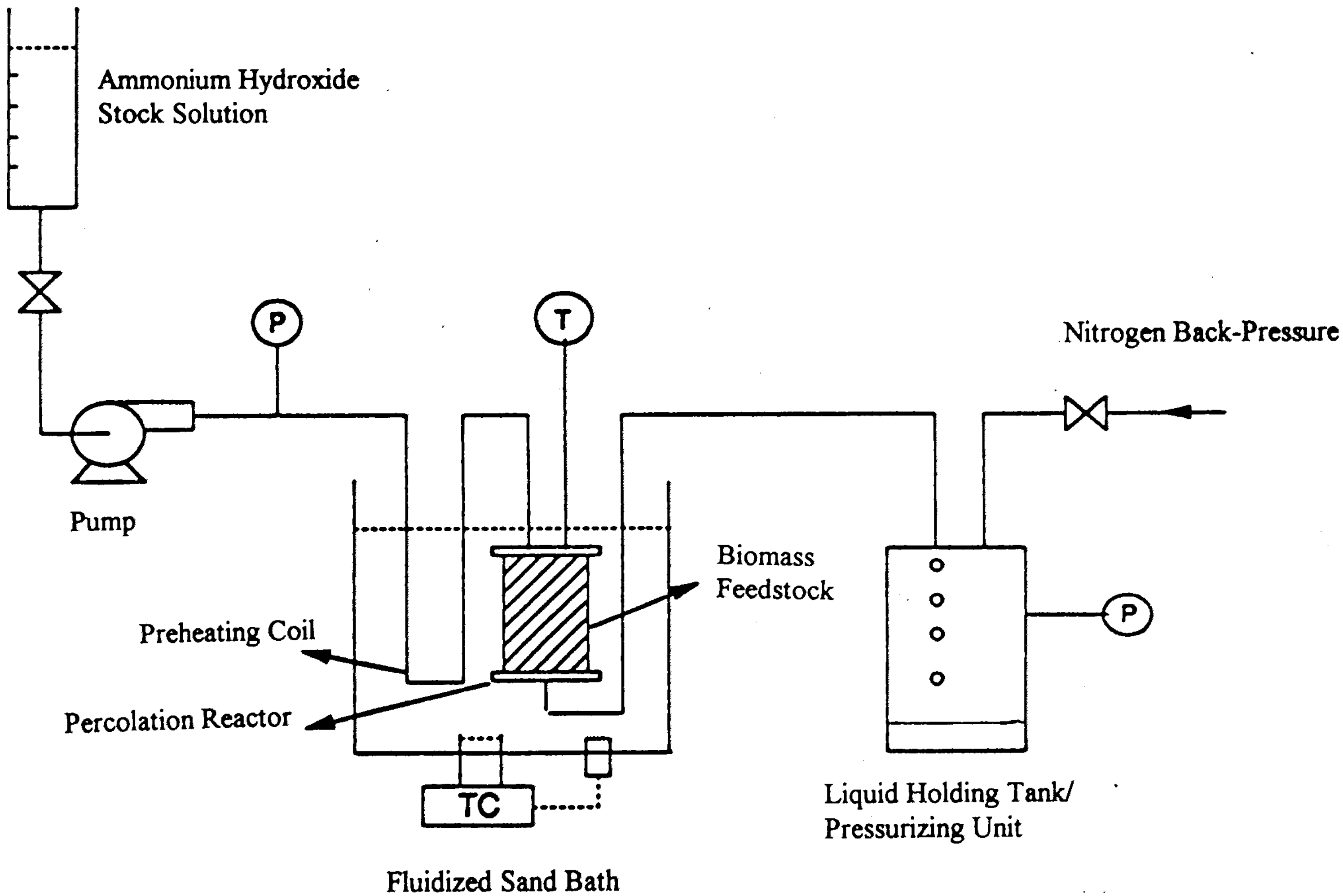


Figure 1. Schematic of an Ammonia Percolation Reactor System.

to remove air absorbed in the wood pores. The biomass slurry was then packed into a reactor. At the conclusion of a reaction, the reactor was cooled to room temperature, the reactor pressure was released, and the slurry sample was transferred into a glass beaker. The pretreated samples were washed with water and filtered to moisture content of 70-80%. A portion of the wet solid residues was oven dried at 105°C overnight for the calculation of weight remaining and the analysis of solid chemical composition. The remaining solid residues was stored in refrigerator at 4°C for enzymatic hydrolysis and/or simultaneous saccharification and fermentation. The liquid effluent collected in a holding tank was transferred into an air-tight glass bottle. The color of the liquid effluent were observed to monitor the progress of pretreatment.

Enzymatic Hydrolysis

Batch hydrolysis was performed at 50°C on a shaker bath agitated at 150 rpm. Phosphate buffer (pH 5.0) was chosen over citrate for improved resolution in HPLC analysis. Unless specified otherwise, for 1 g of biomass (dry basis), 50 ml buffer and 0.3 ml of cellulae enzyme (Cytolase CL), equivalent to 30 IFPU, were added. Samples were taken periodically and analyzed for glucose concentration. Total glucose liberated after 72 hour hydrolysis was analyzed by HPLC and used for calculation of the enzymatic digestibility.

Analytical Methods

The wet biomass sample were weighed out and an aliquot was dried in an oven for 24 hour at 105 °C to determine the exact moisture content. This value used as the initial standard in various weight related calculations including the weight loss after pretreatment reaction. The dried biomass samples were then analyzed for sugar and Klason lignin content. Sugars (glucose and xylose) in solid were analyzed by HPLC using Aminex HPX-87C column following procedures described in NREL-CAT Standard Procedure-#002. Liquid samples from the pretreatment process were analyzed for sugar content after evaporation of ammonia. Klason lignin in solid residue was determined by the procedures described in NREL-CAT Standard Procedure-#003. The liquid samples were acidified by 4% sulfuric acid and autoclaved for one hour at 121°C for secondary hydrolysis and analyzed for sugars by HPLC. The lignin in the liquid stream was determined by spectrophotometric method at 280 nm. Indulin (Westvaco) solution in aqueous ammonia was used as a standard. Surface area of solid was measured by nitrogen adsorption (BET surface area, Quantachrome). Surface structure of solid residue was also observed by Scanning Electron Microscope. The ammonia contents in liquid and solid samples were analyzed by total Kjeldahl nitrogen following the EPA method (600/4-79-020). It was done at Mid-South Testing, Inc., Decatur, AL.

Simultaneous Saccharification and Fermentation (SSF)

Pretreated residual solids were converted to ethanol via SSF procedures described by NREL-CAT Standard Procedure-#008. The SSF experiments were carried out in 250 ml flasks. The flasks contained 5 g of biomass (dry basis) and 100 ml of fermentation broth and were agitated at 150 rpm in shaker incubator at 38°C. The cellulase enzyme loading was approximately 25 IFPU/g of cellulose for these experiments. Liquid samples were taken daily and ethanol content was analyzed by gas chromatography using isopropanol as the internal standard.

Results and Discussion

The initial task of the ARP experiments was to assess its overall effectiveness as a pretreatment and to identify proper operating condition for the process. Typical reaction conditions tested in this study are given in Table 1. The pretreated solid samples were subjected to enzymatic hydrolysis. The simultaneous saccharification and fermentation (SSF) test on the pretreated sample from some of the ARP runs was also carried out.

ARP process

The effect of ammonia concentration, reaction temperature and reaction time on the ARP pretreatment performance was studied. Concentration of ammonia solution was varied between 2.5 and 20.0 wt% at two different reaction temperatures of 160°C and 175°C. Table 2 summarizes the composition data for solid residue after pretreatment. Shown in Figure 2 are the enzymatic digestibility along with the glucose yield after 72 hours of enzymatic hydrolysis as a function of ammonia concentration. Untreated biomass were included in this test as reference. Glucose yield was defined as a percentage of glucose in original biomass that was recovered by enzymatic hydrolysis of pretreated biomass. Enzymatic digestibility was calculated as the percentage of glucose released from pretreated biomass sample by enzymatic hydrolysis. Since small amount of glucose is lost during pretreatment, the glucose yields were lower than enzymatic digestibility values in all cases. It is clearly seen in Figure 2 that the ARP samples indeed exhibit enhanced enzymatic digestibility of cellulose. AT 160°C, the enzymatic digestibility increased with the ammonia concentration. At 175°C, however, the cellulose digestibility increased with the ammonia concentration only up to 10.0 wt% level. Under these experimental conditions, the cellulose digestibility ranged from 63% to 87%. During the ARP pretreatment, about 20-30% of biomass solid was solubilized. Lignin and hemicellulose accounted for the major part of the weight loss. As shown in Table 2, the amount of lignin removed increased as the ammonia concentration varied from 2.5 to 20 wt%. The maximum of lignin loss in these runs was 44%. As high as 36% of hemicellulose was extracted into the reagent liquid during pretreatment process. However, less than 9% of the total glucose content in the original biomass was extracted during the pretreatment.

In the next series of runs, the pretreatment reaction temperature was varied between 150 °C

Table 1. Typical ARP Reaction Conditions

Reaction Conditions	Range
Temperature	150 - 200C
pressure	175 - 325 psi
Reactor volume	33 cc
Sample Size	5 g
Time	1 - 4 hour
Ammonia concentration of pretreatment reagent	2.5 -20 wt%
Reagent flow rate	0.5 -3 ml/min.

Table 2. Effect of Ammonia Concentration on the Composition of Solid Resudues in ARP.

Pretreatment condition: 1 hour, reagent flow rate = 1.0 ml/min.
Enzymatic hydrolysis condition: 30 IFPU/g-treated biomass, 50C, pH 5.0.

Pretreatment Condition Ammonia(wt%)	Temp.(C)	% wt remaining	% Lignin Content	% Glucan Content	% Xylan Content	% Digestibility (72 hr)
Untreated Biomass		100.0	26.0	42.9	14.8	7.8
2.5	160	77.5	22.7	40.9	13.7	62.7
5.0	160	75.5	21.1	41.0	13.4	73.3
10	160	72.5	15.4	40.5	12.8	78.2
20	160	70.7	14.6	39.5	12.5	83.8
2.5	175	75.1	21.1	39.6	11.6	78.9
5.0	175	73.0	18.2	40.9	9.7	78.5
10	175	69.2	15.3	41.3	9.5	87.0
20	175	68.5	15.2	41.8	10.6	81.4

Note: all sugars and lignin content in the table based on oven-dry untreated biomass.

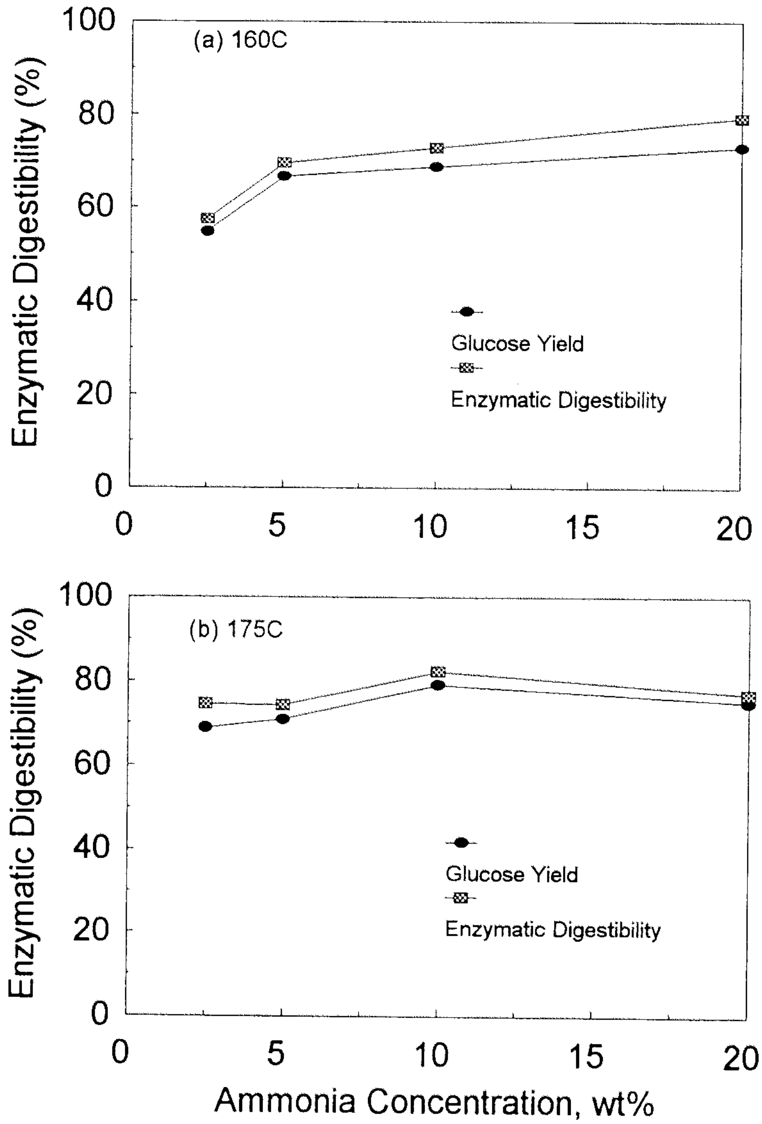


Figure 2. Effect of Ammonia Concentration on Enzymatic Digestibility.

Pretreatment condition: reaction time = 1 hour
reagent flow ate = 1.0 ml/min.
Enzymatic hydrolysis condition: 18 IFPU/g-treated biomass, 50C, pH 5.0.

to 190 °C, while keeping ammonia (10 wt%) reaction time (1 hour) constant. Table 3 summarizes the composition data for solid residue after pretreatment. Shown in Figure 3 are the enzymatic digestibility and the glucose yield after 72 hours of enzymatic hydrolysis as a function of pretreatment reaction temperature. The results collectively indicate that the reaction temperature has indeed a significant effect on the pretreatment. As reaction temperature increases, the enzymatic digestibility and the glucose yield generally increases. The maximum of the observed enzymatic digestibility was 95%. However, at 180°C, the glucose yield is seen to decrease due to significant loss of glucose during pretreatment stage. This was more evident at 190°C in which as high as 14% of glucose were extracted from the biomass during pretreatment. At 180°C and 170°C, the sugar loss was somewhat lower, 6.8% and 3.7%, respectively.

In most of this study, the reaction time of 1 hour was applied. Further increase in digestibility applying reaction time longer than 1 hour was rather insignificant as seen in Figure 4. The enzyme loading in the experiment of Figure 4 was 18 IFPU/g dry biomass. The experimental results gathered to this point suggest that 180°C, 1 hour of reaction time, and 10wt% ammonia represent a near optimal set of pretreatment condition for the ARP process. The enzymatic digestibility of the solid residue pretreated at this condition was found to be 95%, and the overall glucose yield (on the basis of glucose content in the original biomass) was 89%.

Shown in Figure 5 are the data on ethanol yield during simultaneous saccharification and fermentation (SSF) of biomass sample pretreated at 180°C, 1 hour, and 10 wt% ammonia. About 70% of theoretical ethanol yield (based on the cellulose content in pretreated biomass) was obtained after 4 days of SSF. Figure 6 shows the enzymatic digestibility and composition changes at various reaction temperature. The results in this figure indicate that the extent of delignification and xylan extraction are the key factors controlling the effectiveness of pretreatment. The general trend was that biomass samples with lower lignin and xylan content (after pretreatment) exhibited higher enzymatic digestibility. To reaffirm this, digestibility data obtained from various ARP experiments were plotted against the amount of lignin and xylan removed for each sample respectively (Figure 7). Despite the scatteredness of the data, the results of this plot were rather convincing that correlation does exist between the lignin and xylan removal and the digestibility. The figure also shows that enzymatic digestibility increases with the lignin and xylan removal, and then levels off at about 15% xylan and 20% lignin removal respectively. The primary function of the ARP process is

Table 3. Effect of Pretreatment Temperature on the Composition of Solid Residues and Enzymatic Digestibility.

Pretreatment condition: 1 hour, reagent flow rate = 1.0 ml/min.
Enzymatic hydrolysis condition: 30 IFPU/g-treated biomass, 50C, pH 5.0.

Pretreatment Temperature (C)	% wt Remaining	% Lignin Content	% Glucan Content	% Xylan Content	% Digestibility (at 72hr)
Untreated Biomass	100.0	26.0	42.9	14.8	7.8
150	84.7	18.7	42.0	12.4	70.6
160	76.5	15.4	41.0	11.2	77.2
170	70.9	15.6	41.3	10.4	81.9
180	68.9	13.2	40.0	8.8	95.2
190	69.8	13.1	37.0	7.7	95.2

Note: all sugars and lignin content in the table based on oven-dry untreated biomass.

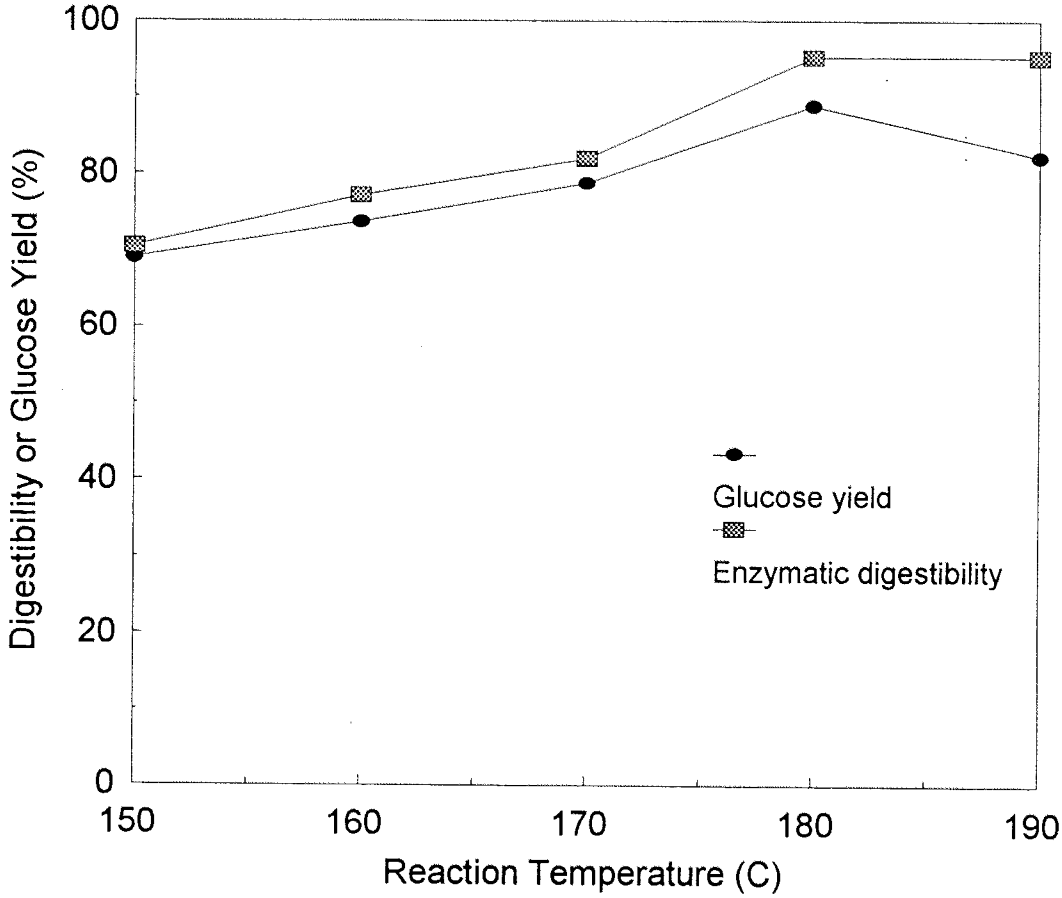


Figure 3. The Effect of Pretreatment Reaction Temperature on Digestibility and Sugar Yield.

Pretreatment conditions: reaction time=1 hour
reagent flow rate=1.0 ml/min.
ammonia concentration=10wt%

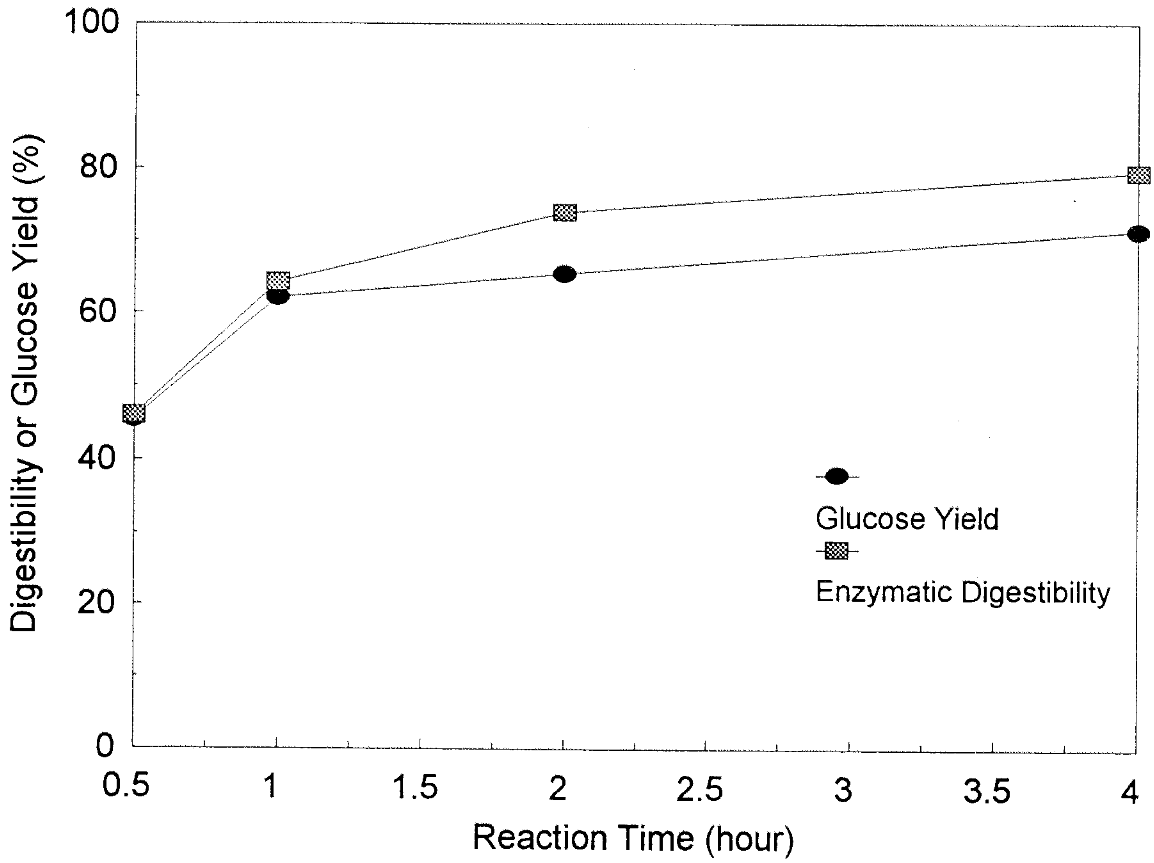
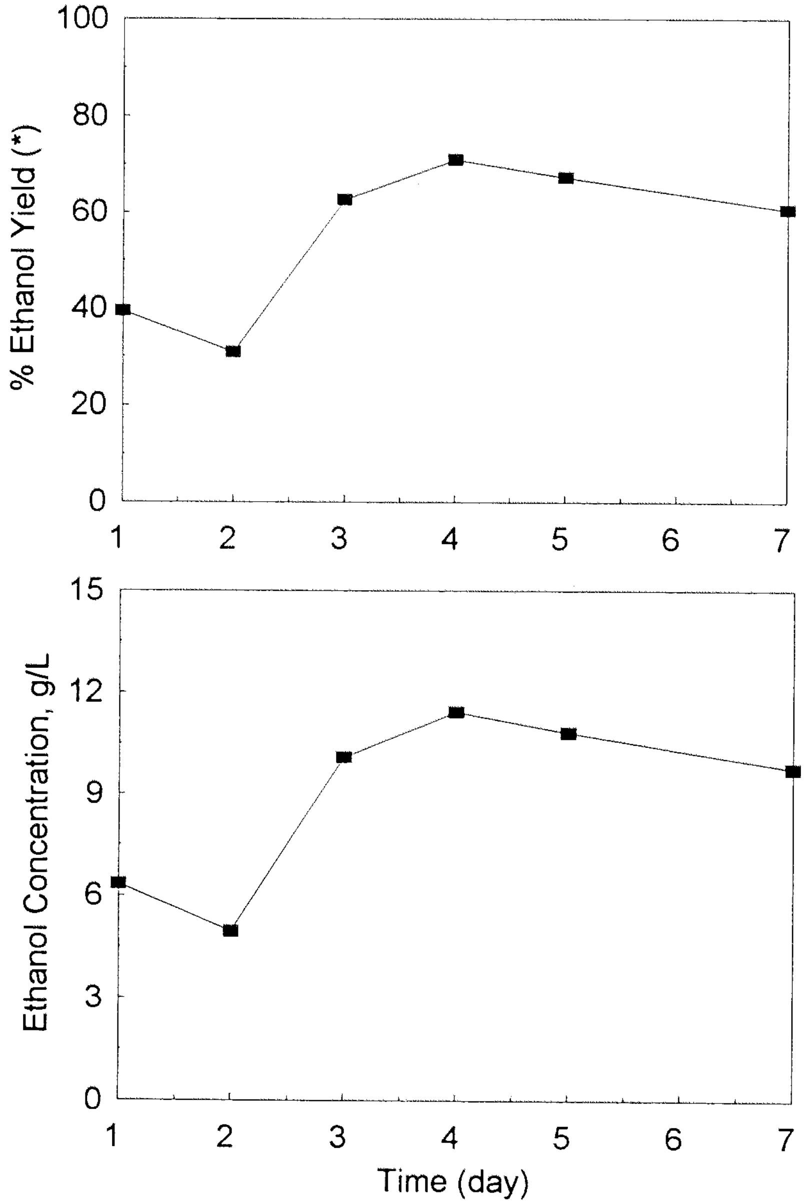


Figure 4. The Effect of Pretreatment Reaction Time on Digestibility and Sugar Yield.

Pretreatment conditions: reaction temperature=175C
reagent flow rate=1.0 ml/min.
ammonia concentration=10wt%



* : calculated from the formula given in NREL procedure #008
(based on the cellulose content in treated biomass)

Figure 5. Concentration and Yield of Ethanol during Simultaneous Saccharification and Fermentation (SSF) of Pretreated Biomass.

Pretreatment conditions: 180C, 1 hr, 10wt% ammonia.
SSF conditions: 5 g dry pretreated biomass/100ml working volume,
25 IFPU/g cellulose in biomass.

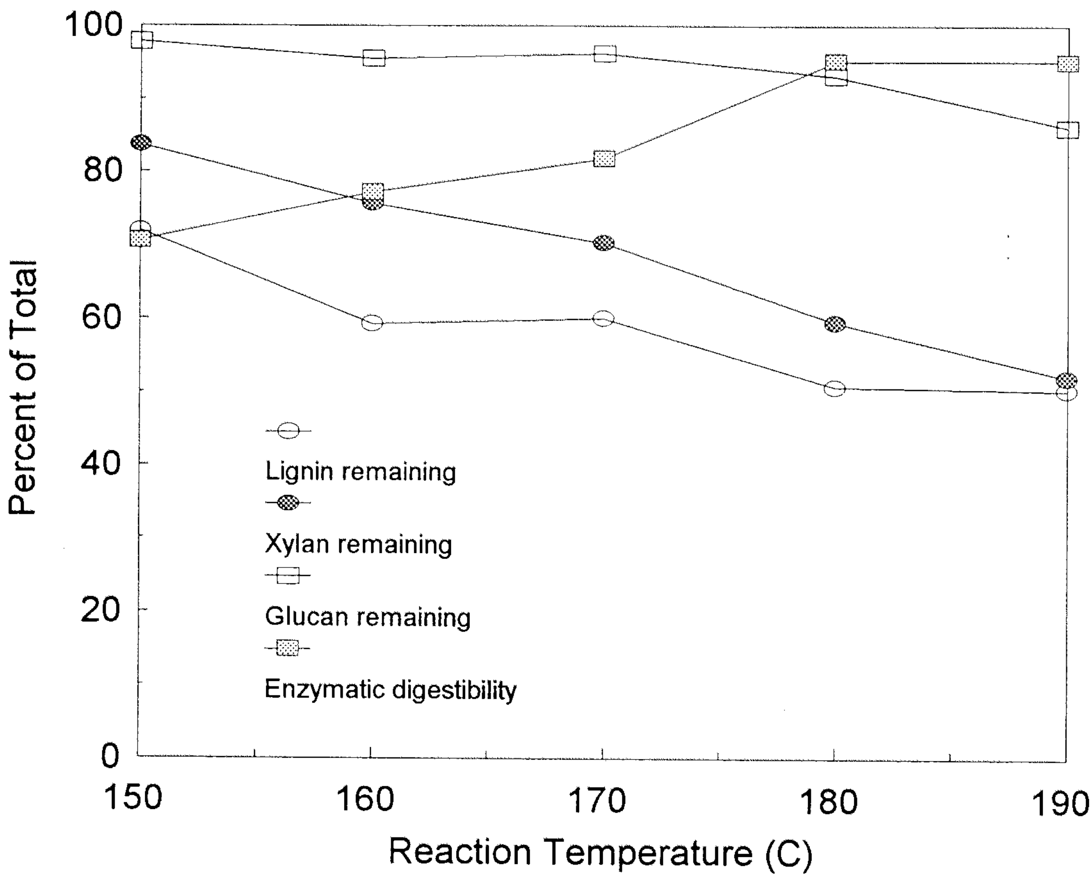


Figure 6. Compositional Changes and Enzymatic Digestibility in ARP.

Pretreatment condition: reagent flow rate=1.0 ml/min.
ammonia concentration=10wt%
Enzymatic hydrolysis condition: 30 IFPU/g-treated biomass, pH 5.0, 50C.

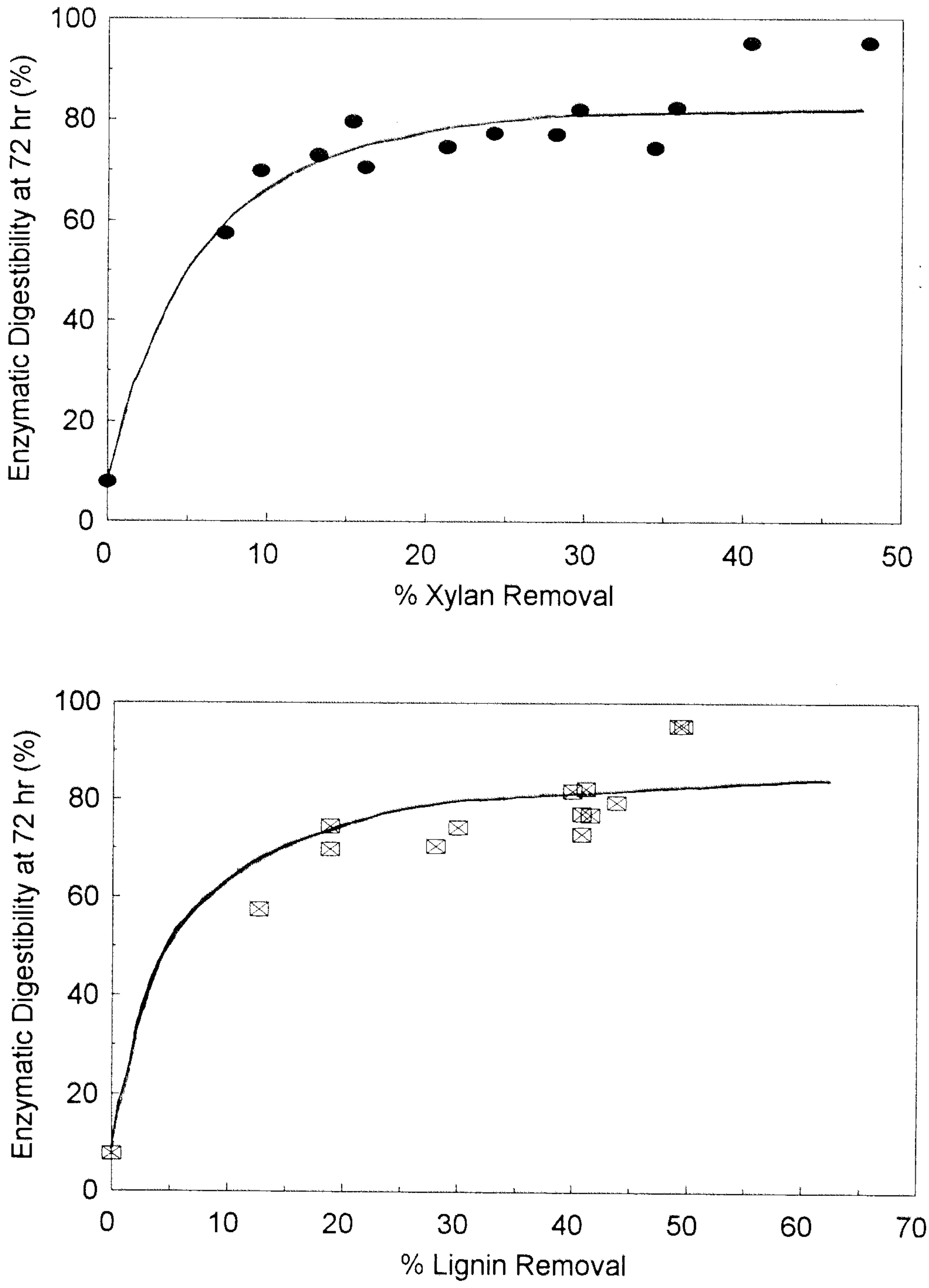


Figure 7. Enzymatic Digestibility vs Degree of Delignification and Percent Xylan Removal in ARP.

delignification. However, it seems inevitable that substantial portion of the hemicellulose is also solubilized during the process. Under the aforementioned operating condition, about 50% of the total Klason lignin and 40% of total xylan in the biomass was extracted out during the pretreatment process.

The solid samples have been observed by scanning electronic microscope (SEM). The morphological changes especially the increase in pore size and porosity were quite evident for the pretreated samples (Figure 8).

In order to examine the changes of accessible surface area upon ARP pretreatment, the BET surface area was measured for the treated and untreated biomass samples. The BET surface area of the biomass sample pretreated at a representative condition (175°C, 1 hour, 10 wt% ammonia) was measured to be 1.2 m^2/g, whereas that of untreated sample was 0.8 m^2/g.

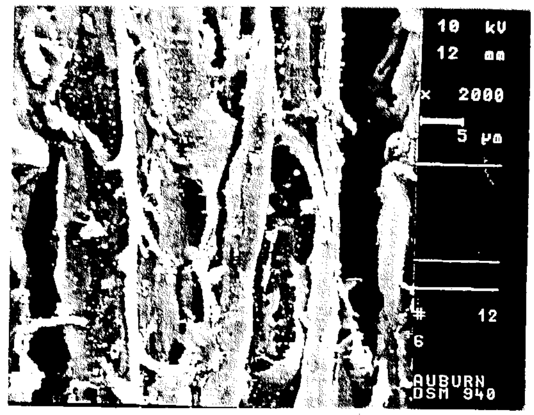
Composition analysis of liquid effluent was also carried out. Difficulties arose with regard to identification and quantification of various components because of overlapping in the current HPLC method. Work is still in progress along these lines.

Enzymatic Hydrolysis of ARP Samples

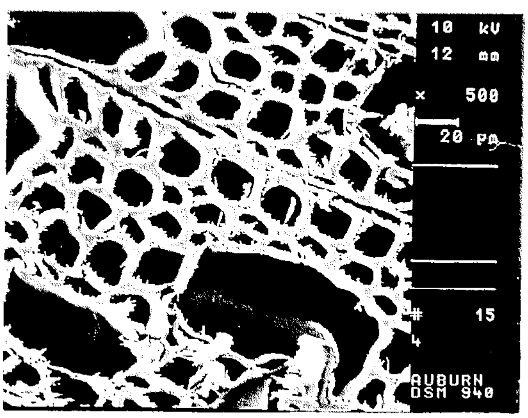
The effect of drying of pretreated biomass was examined. One of the samples from the pretreated runs was divided into three portions. One fraction was oven dried at 105°C overnight, second fraction was kept in distilled water, and the third fraction was washed with NaOH solution before enzymatic hydrolysis. The enzymatic digestibility data for these sample are presented in Figure 9. It is quite clear from the digestibility that drying of the pretreated biomass brings about a detrimental effect on the digestibility, reducing it by about 30%, perhaps due to recrystallization of cellulose in the biomass. Additional washing of biomass with NaOH showed little effect on the digestibility.

The effect of enzyme loading on the glucose yield in the enzymatic hydrolysis of the ARP samples was then investigated. Figure 10 shows the enzymatic digestibility as a function of reaction time under various enzyme loadings. The enzymatic hydrolysis was performed at 50 °C and pH 5.0 with two different enzyme loadings of 15 IFPU (normal loading) and 30 IFPU (double loading) per g-dry biomass. The enzyme used was Cytolase CL, 100 IFPU/mL, supplied by Environmental Biotechnology. In order to examine the enzyme deactivation, additional enzyme of 15 IFPU was

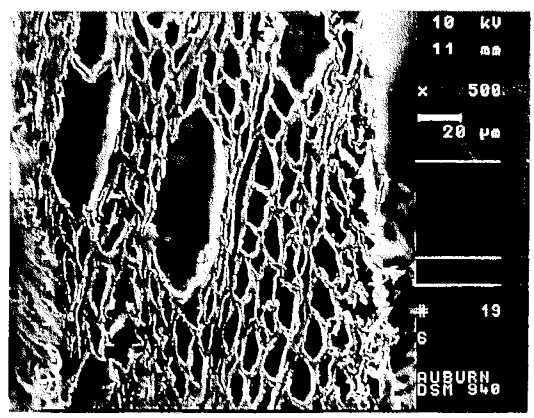
(A) Untreated Biomass (side view)



(B) Pretreated biomass (side view)
(10% ammonia, 175C, 1hr)

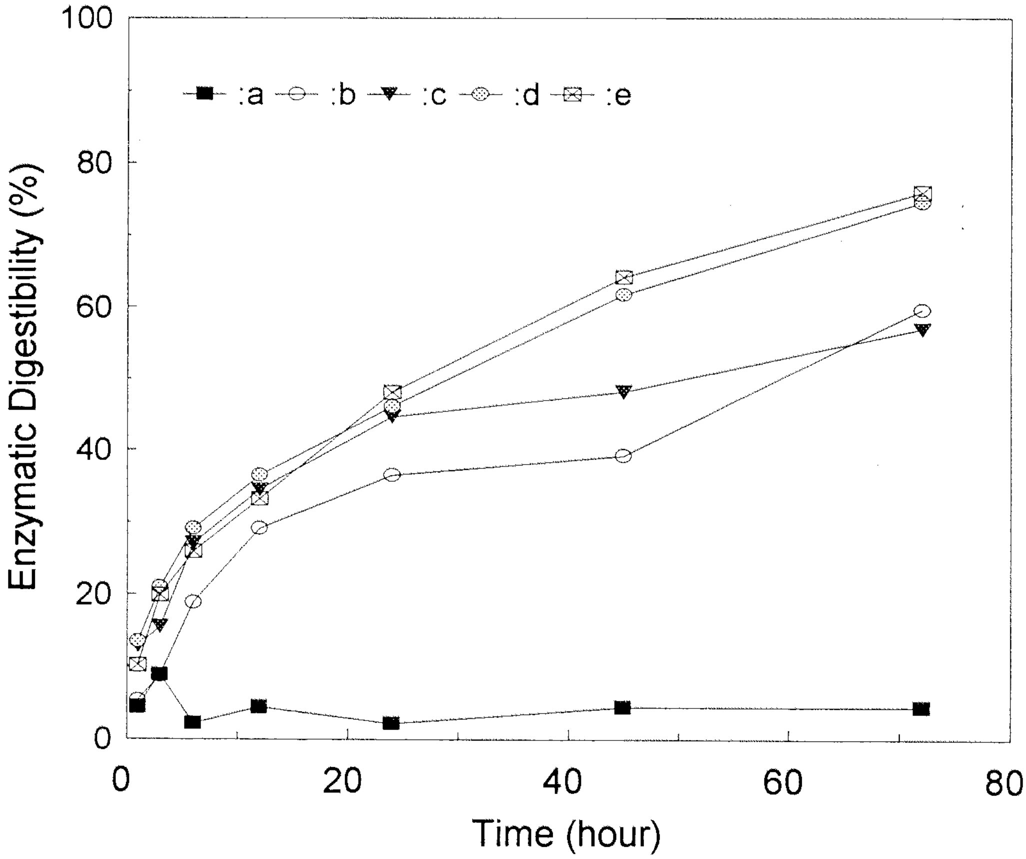


(C) Untreated biomass
(cross sectional view)



(D) Pretreated biomass
(cross sectional view)
(10% ammonia, 175C, 1hr)

Figure 8. SEM Micrographs of Various Biomass Samples.

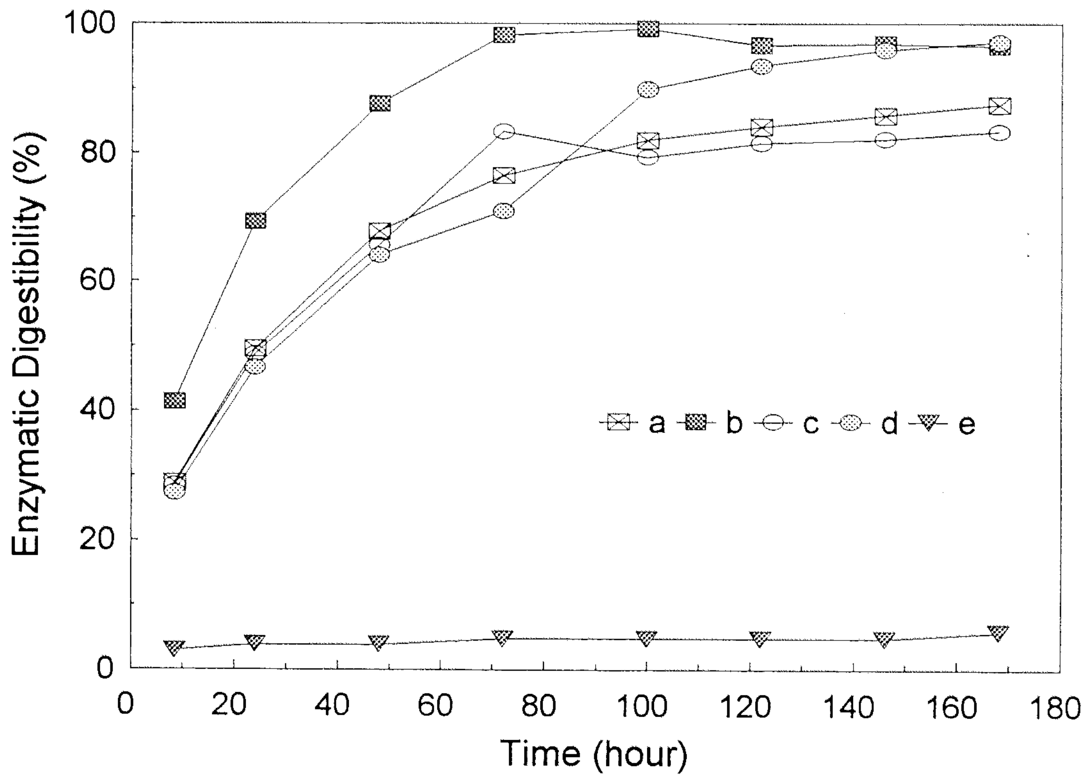


a: untreated
c: pretreated wet sample washed with NaOH
e: untreated filter paper

b: pretreated and oven dried
d: pretreated wet sample soaked in water

Figure 9. Enzymatic Hydrolysis of Various Biomass Samples: Untreated, Pretreated, and Filter Paper.

Pretreatment condition: 175 C, 10wt% ammonia, flow rate=2.0 ml/min.
4 hours of reaction time.
Enzymatic hydrolysis condition: 12.5 IFPU/g-treated biomass, ph5.0, 50C.



a: treated sample with normal loading (15 IFPU/g-biomass)
b: treated sample with double loading (30 IFPU/g-biomass)
c: treated sample with normal loading + B-glucosidase (15 units)
d: treated sample with normal loading and add enzyme (15 IFPU) at 72 hr
e: untreated sample with normal loading

Figure 10. Enzymatic Hydrolysis of Ptretreated Biomass at Different Enzyme Loadings.

Pretreatment condition: 175 C, 2 hr, 10wt% ammonia, flow rate=1.0 ml/min.
Enzymatic hydrolysis condition: 50 C, pH 5.0, (phosphate buffer),
1.0 biomass / 50 ml buffer.

added at 72 hour of enzymatic hydrolysis. The effect of β-Glucosidase supplementation was also studied. The biomass tested in this study was a sample pretreated at 175 °C, with 10 wt% ammonia, for 2 hour.

As seen in Figure 9, double loading rendered about 20% increase in digestibility at the point of 72 hour. However, at 120 hour, there was only 10% increase over that of normal loading. It was also found that the sequential addition of enzyme (15 IFPU at time=0, then additional 15 IFPU at 72 hour) brought about the glucose yield essentially same as that of double loading. Supplementation of β-Glucosidase, however, showed no discernible effect on the glucose yield, probably due to sufficient β-Glucosidase activity in Cytolase CL. From this result, we have decided to use the enzyme loading to 30 IFPU/g biomass in the enzymatic hydrolysis experiments.

Lignin Separation from Spent Liquid

Tests were conducted to see how effectively the lignin as well as ammonia can be separated from the spent liquid. The liquid residue collected from a pretreatment run was evaporated from 80 ml to 40 ml. During the process the pH has changed from the initial value of 11.5 to 7.0, a positive proof that most of ammonia has been evaporated. The resulting liquid (thin slurry) was then centrifuged at 3600 rpm for 10 minutes. The supernatant was poured out and the precipitate was dried at 105 °C overnight, and weighed (to be 0.421g). We believe most of the precipitate is lignin. Lignin is perhaps the only component in the ARP effluent that has pH dependent solubility. During the pretreatment (175 °C , 1 hr, 5wt% NH_3), 0.480 g of lignin (Klason), equivalent to 40% of lignin content in biomass feed, was extracted from 4.6 g of biomass feed (dry basis) into the liquid effluent. Hence, 88% (0.421/0.480) of the dissolved lignin is separated out from the liquid effluent simply by ammonia evaporation and centrifugation. The lignin generated in this process, although subject to further evaluation, is considered to be of high quality because it is free of sulfur or sodium. The delignified liquid effluent may be recycled after ammonia supplementation.

Ammonia Consumption

The consumption of ammonia, if any, is a critical issue for the ARP process from an economic standpoint. The overall material balance of ammonia for the process as determined from one run is as follows. For one gram of dry biomass, 26 ml of 10 wt.% ammonia solution was introduced into the percolation reactor in a semi-continuous manner, the solid being stationary and liquid effluent passing through it. The total ammonia throughput is therefore 2.6 g/g dry biomass. Of this quantity, 0.016 g of ammonia is left in the liquid stream unrecovered after boiling. Most likely most of ammonia exists as ammonia acetate. Additional 0.003 g of ammonia was found to be affixed to the treated solid residue as the result of pretreatment. The net consumption of ammonia is 0.017g/g-dry biomass. The recovery factor of ammonia by simple boiling of the treated liquid effluent is calculated to be 99.3%. One of the key claims of the ARP process is hereby proven that ammonia consumption is extremely low although the throughput of it is high. A possibility also exists that additional steam stripping of the liquid may further increase the recovery factor. The detail calculation and the original data sheet for Kjeldahl analysis for the ARP solid and liquid samples are attached in the Appendix.

Pretreatment with Pure Water

It has been confirmed and pointed out as one of potential problems associated with ARP that quite significant amount of xylan (as high as 40% of total initial xylan) is extracted out during the ARP process. It became of our interest to see if it is feasible to extract all of the xylan content during the pretreatment process. Since the ARP process alone is not satisfactory for that purpose, a two-stage pretreatment process - combination of water hydrolysis and the ARP - was investigated as one of methods to remove and recover all of the xylan fraction from the biomass.

The biomass feed was pretreated with pure water alone, with 10wt% ammonia solution alone, pure water followed by 10wt% ammonia solution, and finally 10wt% ammonia solution followed by pure water. The pretreated samples were enzymatically hydrolyzed. Table 4 summarizes the composition data for solid residue after pretreatment and the enzymatic digestibility. The results indicate that pure water treatment increases xylan extraction from the biomass. The primary function of ARP, on the other hand, is delignification and subsequent increase of digestibility. It is particularly noteworthy that when the biomass was pretreated with pure water at 180°C for 1 hour in straight batch mode (without percolation), 85% of xylan content, but only 5% of glucan content was extracted, an excellent selectivity (Run No. 5). We believe it is due to the fact that the acid (acetate) formed during the batch process is accumulated in the reactor thus increasing the acidity.

Table 4. Effect of Water and Ammonia Pretreatment on the Composition of Solid Residues and Enzymatic Digestibility.

Pretreatment condition: 1 hour, reagent flow rate = 1.0 ml/min.
Enzymatic hydrolysis condition: 30 IFPU/g-treated biomass, 50C, pH 5.0.

Run Number	Pretreatment Condition	% wt remaining	% Lignin Content	% Glucan Content	% Xylan Content	% Digestibility (at 72 hr)
	Untreated Biomass	100.0	26.0	42.9	14.8	7.8
1	ARP (10% ammonia, 180C, 1 hr)	68.9	13.1	40.0	7.7	95.2
2	AHP (water, 180C, 1 hr)	66.1	18.3	41.7	3.4	69.8
3	AHP + ARP (180C, 0.5hr, each, 10% NH3)	62.8	15.7	37.9	5.7	95.0
4	ARP + AHP (180C, 0.5hr, each, 10% NH3)	67.0	14.6	40.6	8.0	84.3
5	AHB (water, 180C, 1hr)	71.1	20.4	40.7	2.2	71.7

Note: all sugars and lignin content in the table based on oven-dry untreated biomass.
ARP: Ammonia Recycled Percolation.
AHP: Auto-Hdrolysis run in Percolation mode.
AHB: Auto-Hydrolysis run in Batch mode.

As the acid concentration increases, hydrolytic reactions become favorable and more of the hemicellulose is hydrolyzed into water soluble oligomers. Treatment with water alone (without the ARP treatment), however, rendered the enzymatic digestibility of only 72%. Results of Table 4 confirm that ammonia pretreatment is indeed a necessary component in raising the enzyme digestibility of the biomass beyond the 70% level. From these results, we feel that the ARP treatment when it is applied in conjunction with pure water batch would eventually prove to be a highly efficient pretreatment method, one that will ensure selective separation of xylan fraction from the biomass as well as sufficient delignification to give acceptable degree of enzymatic digestibility.

Appendix

- The Original Data Sheet for Kjeldahl Analysis of Nitrogen of the ARP Solid and Effluent.
- The calculation of ammonia consumption.

MID–SOUTH TESTING, INC.

2220 BELTLINE ROAD, SOUTHWEST • DECATUR, ALABAMA 35601 • (205) 350-0846

Y.Y. LEE
AUBURN UNIVERSITY
DEPARTMENT OF CHEMICAL ENGINEERING
230 ROSS HALL
AUBURN AL 36849-5127

LABORATORY NUMBER	SEE BELOW
DATE RECEIVED	06-16-93
SHIPPING MODE	U.S. MAIL
SAMPLE DATE	NOT INDICATED
PARAMETER	TOTAL KJELDAHL NITROGEN
METHOD	351.3

LABORTORY NUMBER	SAMPLE TYPE	ANALYSIS
93-9745 B	# 1	3463 ppm
93-9746 C	# 2	1524 ppm
93-9747	# 3 (SLURRY)	1535 ppm

Note:

#1 Retreated Sample
#2 Untreated Sample
#3 ARP Effluent (after boiling)

ANALYZED BY EPA APPROVED METHODS

*1 EPA METHODS FOR CHEMICAL ANALYSIS OF WATER AND WASTES
600/4-79-020, MARCH 1979

ANALYST: SHARON CHYNOWETH
DATE/TIME: 06-23-93/0800

Calculation of Ammonia Consumption

11.85 g biomass was pretreated with 10wt% ammonia in an ARP process, 307 ml ammonia solution was pumped through the reactor, and finally 8.995g treated biomass and 100.62 g ARP effluent after boiling were obtained.

Total untreated biomass = 11.85 g
10wt % ammonia supplied = 307 ml
Total treated biomass = 8.995 g
ARP effluent after boiling = 100.62 g

Nitrogen content (g Nitrogen/ g-sample):
#1 Pretreated biomass = 0.003463
#2 Untreated biomass = 0.001524
#3 ARP effluent (after boiling) = 0.001535

Basis: 1 g untreated biomass.

1. The total ammonia throughput:
   307*10%/11.85=2.591g

2. Ammonia in effluent:
   100.62*0.001535/11.85 = 0.013 g nitrogen
   = 0.016 g ammonia (NH_3)

3. Ammonia in treated solid residue:
   8.995*0.003463/11.85 = 0.0026 g nitrogen
   = 0.003 g ammonia

4. The total ammonia consumption:
   (100.62*0.001535 + 8.995*0.003463 - 11.85*0.001524)/11.85 = 0.014 g nitrogen
   = 0.017 g ammonia

5. Ammonia recovery rate:
   (2.591-0.017)/2.591*100 = 99.3%